Published in final edited form as:

Infect Genet Evol. 2010 April ; 10(3): 386–396. doi:10.1016/j.meegid.2009.04.014.

Extensive survey on the prevalence and genetic diversity of SIVs in primate bushmeat provide insights into risks for potential new cross-species transmissions

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Abstract

To evaluate the risk of cross-species transmissions of SIVs from non-human primates to humans at the primate/hunter interface, a total of 2586 samples, derived from primate bushmeat representing 11 different primate species, were collected at 6 distinct remote forest sites in southeastern Cameroon and in Yaoundé, the capital city. SIV prevalences were estimated with an updated SIV lineage specific gp41 peptide Elisa covering the major part of the SIV diversity. SIV positive samples were confirmed by PCR and sequence analysis of partial *pol* fragments. The updated SIV Elisa showed good performance with overall sensitivity and specificity of 96% and 97.5% respectively. The overall SIV seroprevalence was low, 2.93% (76/2586) and ranged between 0.0% and 5.7% at forest sites, and reached up to 10.3% in Yaoundé. SIV infection was documented in 8 of the 11 species with significantly different prevalence rates per species: 9/859 (1.0%) in *Cercopithecus nictitans*, 9/864 (1.0%) *Cercopithecus cephus*, 10/60 (16.7%) *Miopithecus ogouensis*, 14/78 (17.9%) *Colobus guereza*, 15/37 (40.5%) *Cercopithecus neglectus*, 10/27 (33.3%) *Mandrillus sphinx*, 6/12 (50%) *Cercocebus torquatus*, and 3/6 (50%) *Chlorocebus tantalus*. No SIV infection was identified in *Cercopithecus pogonias* (n=293), *Lophocebus albigena* (n=168) and *Cercocebus agilis* (n=182). The SIV prevalences seem to vary also within species according to the sampling site, but most importantly, the highest SIV prevalences are observed in the primate species which represent only 8.5% of the overall primate bushmeat. The phylogenetic tree of partial *pol* sequences illustrates the high genetic diversity of SIVs between and within different primate species. The tree showed also some interesting features within the SIVdeb lineage suggesting phylogeographic clusters. Overall, the risk for additional cross-species transmissions is not equal throughout southern Cameroon and depends on the hunted species and SIV prevalences in each species. However, humans are still exposed to a high diversity of SIVs as illustrated by the high inter and intra SIV lineage genetic diversity.

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Nucleotide sequence accession number

The new SIV sequences reported in this study are available in GenBank under the following accession numbers: (Accession numbers pending).

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Keywords

SIV; HIV; AIDS; Non-human primates; prevalence; cross-species transmission; genetic diversity

INTRODUCTION

HIV-1 and HIV-2, the etiologic agents for AIDS in humans are the results of cross-species transmissions of lentiviruses from non-human primates (Hahn et al., 2000). The closest simian relatives of HIV-1 are SIVcpz and SIVgor, from chimpanzees (*Pan troglodytes troglodytes*) and gorillas (*Gorilla gorilla gorilla*) in West central Africa (Gao et al., 1999; Sharp et al., 2005; Keele et al., 2006; Van Heuverswyn et al., 2006), and HIV-2 is derived from SIVsmm from sooty mangabeys (*Cercocebus atys*) in West Africa (Hirsch et al., 1989; Gao et al., 1992). SIVsmm has been transmitted to humans at least 8 times in West Africa, and similarly, HIV-1 group M, N and O are each the result of an independent cross-species transmission of SIVs from the SIVcpz/SIVgor lineage (Hahn et al., 2000; Sharp et al., 2005; Van Heuverswyn et al., 2006). Currently, serological evidence of SIV infection has been shown for 40 different primate species and has been confirmed by sequence analysis in 32 species. Complete SIV genome sequences are available for 20 species (VandeWoude and Apetrei, 2006; Van Heuverswyn and Peeters, 2007; Locatelli et al., 2008a, Liegeois et al., 2009). A high genetic diversity is observed among the different SIVs, but generally each primate species is infected with a species-specific virus, which forms monophyletic lineages in phylogenetic trees. Among these monophyletic lineages, there are examples of co-evolution between viruses and their primate hosts, but there are also many examples of cross-species transmissions followed or not by recombination between distant SIVs, and one species can even harbour two different SIVs (Hahn et al., 2000; Bibollet-Ruche et al., 2004; Aghokeng et al., 2007).

The emergence of HIV-1 and HIV-2 in the human population is most likely the result of contact with infected blood or tissues through hunting and butchering of non-human primates. In a previous study on primate bushmeat in Cameroon, we showed that the majority of hunted primates is represented by multiple *Cercopithecus* species, colobus monkeys, mandrills, drills, etc... from which a significant proportion was SIV infected, based on cross-reactivity with HIV-1 and HIV-2 antigens (Peeters et al., 2002). Most importantly, this study illustrated the ongoing exposure of humans to a plethora of different SIVs. In the mean time, other studies reported also high rates of ongoing exposure to non-human primates, through hunting, butchering and injuries, in rural villagers in forest sites in Cameroon (Wolfe et al., 2004a). Cross-species transmission of other retroviruses has also been documented among hunters (Mahieux et al., 1998; Wolfe et al., 2004b). It can thus not be excluded that simian lentiviruses from other primate species than mangabeys, chimpanzees and/or gorillas have been or will be transmitted to humans. In order to investigate this, more detailed analysis of SIV prevalence and SIV diversity in wild primate populations as well as sentinel surveys among human populations frequently exposed to primates are needed. For such studies, SIV lineage specific serological assays have been developed, but they need to be regularly updated with the inclusion of additional antigens, as new SIV lineages are discovered (Simon et al., 2001; Ndongmo et al., 2004; Aghokeng et al., 2006).

In this report we used an updated Elisa assay, using synthetic peptides of the gp41 immunodominant region, to study more in detail the SIV reservoir in primate bushmeat in southern Cameroon in order to evaluate the potential risks for cross-species transmissions at the primate/hunter interface. We document SIV prevalences and SIV diversity in samples mainly collected as dried blood spots in different settings in remote forest areas.

MATERIALS AND METHODS

Synthetic gp41 peptides and biotinylated gp41 (bgp41) ELISA

A total of 13 peptides of 24 amino acid residues each, covering the immunodominant region (IDR) of the gp41 transmembrane domain of all major SIV/HIV lineages known at the time we performed the work were synthesized; HIV-1/SIVcpz, HIV-2/SIVsmm, SIVrcm, SIVagm, SIVgsn/mus/mon, SIVdeb, SIVtal, SIVsyk, SIVlho/sun, SIVmnd-2, and SIVwrc. (Table 1). In order to optimize the sensitivity of the assay, the gp41 peptides were biotinylated and purchased from Neosystems (Neosystems, Strasbourg, France). For SIVcol, gp41 peptides could not be synthesized due to their low solubility property and the previously described synthetic peptide corresponding to the V3 loop region of the extracellular envelope domain of this SIV lineage was used to identify corresponding antibodies (Aghokeng et al., 2006). Bgp41 ELISAs were carried out as previously described for synthetic V3 loop ELISA with minor modifications (Aghokeng et al., 2006), mainly corresponding to the coupling phase of streptavidin to biotinylated peptides and each well of polyvinylmicrotiter plates were coated with 0.08 µg of streptavidine coupled biotinylated peptides. Sample preparation from DBS specimens consisted of incubating two 6 mm disks of each dried blood spot (DBS) in 1 ml of hypertonic PBS buffer over night, at room temperature under gentle rotation. One hundred microliter of diluted serum/plasma (1/100) or DBS eluate was added to each peptide-coated well. The cut-off value of a particular bgp41 ELISA was calculated as the mean optical density (OD) for all antibody negative reference sera plus 6 standard deviations (Crowther, 2001; Aghokeng et al., 2006). Performance of each bgp41 ELISA was determined in terms of sensitivity and specificity, calculated for the detection of antibodies of both homologous (same species) and heterologous (different species) viruses.

Non-human primate samples

A total of 145 well-characterized reference plasma samples, for which sufficient plasma was available, were selected from our non-human primate (NHP) reference panel in which SIV infection was either confirmed or ruled out by highly sensitive PCR approaches (Peeters et al., 2002; Aghokeng et al., 2006). The uninfected reference panel included 95 samples from 8 different primate species, while the SIV positive reference panel was composed of 50 samples from 9 different primate species (Table 2).

For SIV prevalence studies among NHP, 1856 samples were collected as dried blood spots on filter paper (NHP DBS) in 5 remote forest sites in southern Cameroon. After approval from the Cameroonian authorities, these samples were collected from 5 geographically distinct sites throughout southeastern Cameroon, from 2001 to 2004 in one site (CS1), and during 2004 in 4 sites (CS2-CS5a) (Figure 1). In practice, whole blood was collected consecutively from fresh primate bushmeat confiscated by the governmental anti-poaching program or by hunters who accepted to collaborate and who were educated about conservation of primates and about the risks associated with direct contact with primate blood, body fluids or tissues. Whole blood was then subsequently spotted onto filter paper, either Whatman (Whatman plc, Kent, UK) or 903 filter paper cards (Schleicher & Schuell, Keene, NH) depending on what was available. Each paper card was first identified with a sample ID, the site and date of sample collection, as well as the corresponding primate species. After air dry at ambient temperature overnight, NHP DBS were wrapped into individual envelopes and stored at ambient temperature until shipment to Yaoundé where samples were centralized for long-term storage also at ambient temperature.

In addition, a total of 730 NHP samples for which whole blood frozen at -20°C was collected on primate bushmeat from a previous study (Aghokeng et al., 2006), were reanalyzed for comparison by geographic location. We previously reported SIV prevalences on these samples

but without taking into account their geographic origin, but 158 samples were collected at CS5b in 2001, 300 were collected in 2000 in central south Cameroon here identified as CS6. Finally, 272 samples were collected on markets in Yaoundé (CS7) and the exact geographic origin of the primates is thus unknown, although they are most likely imported to Yaoundé from south and south-east Cameroon where primate hunting is common.

DNA extraction and PCR amplification of SIVs on antibody positive samples

Total DNA was extracted from NHP dried blood spot samples with the QIAamp blood kit (Qiagen, Courtaboeuf, France) using the manufacturer's instructions with minor changes, mainly consisting of increasing the incubation time during the viral lysis step and therefore increasing DNA release during the extraction process. Briefly, 5 to 8 DBS disks of 6 mm diameter each were incubated in the QIAamp lysis buffer at 56°C for 2 h, instead of 10 min normally applied to conventional whole blood samples. Tubes were vortexed every 30 min during the incubation period to allow adequate sample homogenization. The next steps were strictly performed according to manufacturer's instructions. For whole blood samples, total DNA was isolated from buffy-coat using the QIAamp blood kit according to the kit insert. PCR analyses were performed on SIV antibody positive samples using previously described conditions as well as universal and highly sensitive primers, either SIV strain- or SIV lineage-specific designed in the *pol* or *env* region (Clewley et al., 1998; Courgnaud et al., 2001; Liegeois et al., 2006; Aghokeng et al., 2007). Expected DNA products included fragments of various sizes, ranging from 196 bp to 2050 bp.

Species identification recorded in the field was confirmed on all SIV positive and a subset of negative samples by amplifying a 386 bp mtDNA fragment spanning the 12S rRNA gene using primers 12S-L1091 and 12S-H1478 (Van Der Kuyl et al., 2000).

Phylogenetic analyses

We aligned the newly obtained nucleotide and protein sequences using ClustalX 2.0 (Thompson et al., 1997), and applied minor manual adjustment when necessary. Sites that could not be unambiguously aligned and those containing gaps were excluded. Trees were inferred by the Bayesian method (Yang and Rannala, 1997) implemented in MrBayes 3.1.2 (Huelsenbeck and Ronquist, 2001) using a General Time Reversible (GTR) model with invariable rates for nucleotide sequences and an amino acid substitution matrix for inference of retrovirus and reverse transcriptase phylogeny, the rtREV model with rates equal to invariable. These models were selected as the best-fit evolutionary model for our data sets using ProtTest for protein sequences (Abascal et al., 2005), and Modeltest for nucleotide sequences (Posada and Crandall, 1998). One to 5 million generations were applied to run MrBayes analyses and Bayesian likelihoods and parameters were examined with the Tracer program (<http://tree.bio.ed.ac.uk/software/tracer/>). Newly studied SIV sequences were compared to reference SIV strains obtained from GenBank (<http://www.ncbi.nlm.nih.gov/>).

RESULTS

Performances of the updated SIV lineage specific biotinylated gp41 (bgp41) ELISA on a reference panel

We first evaluated the sensitivity and specificity of homologous but also of heterologous antibody detection on the reference panel. Heterologous antibody detection is essential for the SIV ELISAs, because for several primate species, SIVs have not been identified yet and they can only be identified by cross-reactivity. Table 3 summarizes the sensitivities and specificities for each peptide. For 40 of the 50 positive samples, the homologous bgp41 ELISA was available and 39 samples reacted strongly with their bgp41 peptide counterpart resulting into an overall homologous sensitivity of 97.5%. One talapoin monkey did not react with the SIVtal peptide,

but was also negative in the previously reported recombinant gp41 ELISA (Aghokeng et al., 2006). However, the false negative talapoin sample did not cross-react with any of the heterologous bgp41 peptides, and was therefore scored as negative. Since the SIVcol gp41 peptide was not available, we evaluated only heterologous detection in the 10 colobus samples, and 1 of the 10 SIVcol positive samples did not cross-react with any of the other SIV antigens. Thus, taking together the homologous and heterologous bgp41 reactivities, 48 out of 50 SIV positive samples were identified as positive resulting in 96% sensitivity. Unfortunately, the SIVcol positive reference sample which was not detected by heterologous reactivity with the panel of gp41 antigens, scored also negative to SIVcol-V3 ELISA. Each of the 13 bgp41 ELISAs cross-reacted with at least one sample from a different primate species (data not shown), with SIVmus-bgp41 ELISA showing the highest cross-reactivity by detecting more than 75% of the samples included in the positive reference sample panel.

None of the negative sera tested positive with the homologous bgp41 ELISA, resulting in 100% specificity for homologous antibody detection. However, 2 negative samples reacted weakly (OD/cut-off ratio <2) with a single heterologous bgp41 peptide, resulting in an overall 97.9% specificity of homologous and heterologous antibody detection.

Given the extraordinary SIV diversity, few false negative samples are observed, and our assay has an overall sensitivity of 96% and a specificity of 97.5% with highly cross-reactive capacity, and should thus allow the detection of the majority of SIV infections.

The primate species collected as bushmeat at the different localities

In order to better evaluate the SIV reservoir and exposure to SIVs, samples were collected at different remote forest sites in southeastern Cameroon. A total of 1856 samples were collected as dried blood spots (DBS) at 5 remote forest sites (Figure 1) and represent 11 different primate species; *C. nictitans* (n=673), *C. cephus* (n=647), *C. pogonias* (n=226), *L. albigena* (n=129), *C. agilis* (n=83), *M. ogouensis* (n=37), *C. guereza* (n=25), *C. neglectus* (n=15), *M. sphinx* (n=11), *C. torquatus* (n=7), and *C. tantalus* (n=3). The number of samples of each primate species and at each collection site is shown in Table 4. The proportions of the species differ among sites and correspond to primate species living in the area. Overall, 3 species, *C. nictitans*, *C. cephus*, and *C. pogonias*, predominate in each site because they are widely distributed in southern Cameroon. Mandrills and red-capped mangabeys were only present in the southwest, because their habitat is restricted to this geographic area. Similarly, agile and grey-cheeked mangabeys are only present in the south-central and southeastern sites (Groves, 2001). In order to evaluate whether sample bias could have occurred, we compared the species distribution in the southwest (CS5a, Ebolowa) to the species distribution observed in our previous survey in 2001 in the same area (CS5b). As shown in Table 4, the same species predominate and are represented at comparable proportions (*C. cephus*, *C. nictitans* and *C. pogonias*), but for the minority species, the numbers differ slightly. Interestingly, the global SIV prevalences differed also only slightly, 3.7% (2.2–6.1) and 5.7% (3.5–8.8).

In order to have a better overview of the geographic distribution of SIV prevalences in primate bushmeat we reanalyzed the data on SIV prevalences from our previous study according to the origin of the samples (Aghokeng et al., 2006). In this previous study, samples were also collected in south-central Cameroon (CS6), at the same site in the southwest (CS5b) and on markets in the capital city Yaoundé (CS7). The distribution of primate species in Yaoundé is not representative for the surrounding forest areas because bushmeat arrives in the city essentially by train and road from remote forest areas of the south and east regions. As a consequence, 11 different species were sampled in Yaoundé versus 5 to 8 species only in the other sites.

SIV prevalence in primate bushmeat

The 13 bgp41 SIV and the SIVcol V3 loop Elisa were then used to screen SIV infection on all DBS samples. Table 4 shows the number of SIV positive samples per species and per site for the DBS and also for the whole blood samples from the previous study tested by the recombinant gp41 Elisa (Aghokeng et al., 2006). The overall seroprevalence was low, 2.93% (76/2586), and heterogeneous among the different sites, ranging between 0.0% and 5.7% for the forest sites, with the highest prevalences observed in the southwest. Interestingly, 10.3% of primates were SIV infected on markets in Yaoundé. SIV infection was documented in 8 of the 11 species with significantly different prevalence rates per species. We observed a high proportion of SIV positive individuals for five species, although for some species the sample size was relatively low: 3/6 *C. tantalus* (50.0%), 6/12 *C. torquatus* (50.0%), 15/37 *C. neglectus* (40.5%), 10/27 *M. sphinx* (33.3%), and 10/60 *M. ogouensis* (16.7%) We only identified positive individuals among mantled guerezas (*C. guereza*) in south-central Cameroon (CS6) and on the market in Yaoundé. We did not identify any new SIV infection in crested mona, grey-cheeked and agile mangabeys and found only 1% of *C. nictitans* and *C. cephus* infected with SIV. Interestingly, the highest prevalences among *C. cephus* (7.3%) and *C. nictitans* (7.1%) were observed in south-central Cameroon (CS6) and at markets in Yaoundé, respectively.

The SIV prevalences differ thus among species, and seem to vary also within species according to the sampling site. But most importantly, the highest SIV prevalences are observed in the primate species, which represent only 8.5% of the overall primate bushmeat, and the highest prevalences were observed in southwest and central Cameroon. These data suggest that risk for additional cross-species transmissions is not equal throughout southern Cameroon and depends on the hunted species and most likely also on prevalences in each species, which can differ according to their geographic origin.

Molecular confirmation and characterization of SIV strains from primate bushmeat

In order to confirm SIV infection and document SIV diversity, all ELISA positive DBS and whole blood samples for which sufficient material was available, were subjected to PCR amplification using different sets of universal and highly sensitive primers as well as SIV lineage specific primers. Overall the amplification rate was low on DBS samples, 7 out of 22 only, most likely due to the long and sub-optimal storage of the DBS at room temperature. SIV infection in DBS was confirmed in 4/4 *C. neglectus*, 2/7 *M. ogouensis*, and 1/2 *M. sphinx*. Six samples were successfully amplified in the *pol* region and one of the four De Brazza samples was only amplified in the *env* region with lineage specific primers, and phylogenetic analysis confirmed that this latter sample was a new SIVdeb strain (data not shown). On the whole blood samples, SIV infection was confirmed by PCR and sequence analysis on 34 of the 54 positive samples for the field sites; 5/6 *C. cephus* (Courgnaud et al., 2003; Aghokeng et al., 2007), 11/14 *C. guereza*, 3/3 *M. ogouensis* (Liegeois et al., 2006), 4/10 *M. sphinx*, 6/8 *C. nictitans*, 5/11 *C. neglectus*, 3/3 *C. torquatus* and 0/3 *C. tantalus*. For SIV positive samples collected in Yaoundé (CS7), SIV infection was confirmed in 5 of the 6 SIV positive species; 2/4 *C. neglectus*, 6/8 *C. nictitans*, 8/10 *C. guereza*, 2/2 *M. ogouensis* and 2/3 *M. sphinx*, 0/1 *C. tantalus*. Some of the *C. cephus*, *C. nictitans*, *C. guereza*, *M. ogouensis* and *C. neglectus* samples have been previously described (Courgnaud et al., 2003; Liegeois et al., 2006; Aghokeng et al., 2007;). Figure 2 shows the phylogenetic analyses of the new *pol* sequences of sufficient length identified in this study, together with SIVs identified in previous studies in Cameroon (Peeters et al., 2002). This partial *pol* tree illustrates the high genetic diversity of SIVs between and within different primate species. A high genetic diversity is observed for the SIVmus strains, and previous studies on the full-length genome characterization of this strains showed that indeed, mustached monkeys can be infected with two different SIV lineages, one being a recombinant between SIVmus and SIVgsn from cohabiting greater spot

nosed monkeys (Aghokeng et al., 2007). The tree showed also some interesting features within the SIVdeb lineage since two newly identified SIVdeb sequences (04CMPF3061 and 04CMPF3074) formed a separate clade within the SIVdeb lineage and clustered distantly from the previously described SIVdeb sequences (CM5 and CM40) (Bibollet-Ruche et al., 2004) and the new 04CMPF1122 sample (Figure 2). Since 04CMPF3061 and 04CMPF3074 were collected in the southwest (CS5, Ebolowa) and 04CMPF1122 was collected in the south-central Cameroon (CS3, Nanga Eboko) this could suggest a geographic differentiation of SIVdeb viruses in Cameroon. The exact origin of the previously described SIVdeb strains (CM5 and CM40) is not exactly known, but they were collected as bushmeat in Yaoundé, and were therefore most likely hunted in eastern Cameroon. Since previous studies also described SIVdeb sequences from De Brazza's monkeys originating from Uganda, we performed a maximum likelihood tree including both Ugandan and Cameroonian SIVdeb Pol sequences to assess the clustering pattern of these strains according to their geographic origin (Figure 3). Interestingly, the SIVdeb sequences clustered in three distinct sublineages; one sublineage represented by previously described SIVdeb strains including CM5 and CM40 isolates, a second sublineage represented by Ugandan strains, and a third one involving only the newly characterized SIVdeb sequences, 04CMPF3061 and 04CMPF3074. Although we performed the analysis on short Pol sequences (164 amino-acids), these data support a geographic clustering of SIVdeb strains not only between Cameroonian and Ugandan strains, but also between Cameroonian strains.

DISCUSSION

In this study we analyzed the risk of cross-species transmissions from SIVs from non-human primates to humans at the primate/hunter interface. We screened 1856 samples, derived from primate bushmeat for the prevalence of SIV infections with an updated SIV specific Elisa, representing the majority of the actually known SIV lineages and covering the major part of the SIV diversity. Together with results from our previous investigations, we showed that SIV prevalences differ among species and seem also to vary within species according to the sampling site. We also showed low SIV prevalences among the most frequently hunted primate species and a high genetic diversity between and within SIV lineages.

Validation of the updated SIV specific Elisa

We previously used and validated an ELISA using recombinant proteins from the entire ectodomain of the SIV transmembrane envelope region to detect SIV antibodies in non-human primates (Aghokeng et al., 2006). In this new study we updated the assay by increasing the number of SIV lineages (SIVagm, SIVlho and SIVwrc) and by replacing recombinant gp41 antigens by synthetic peptides. The advantage of synthetic peptides is that they are easy to design and routinely produce compared to recombinant proteins, and can thus be more rapidly included to update the assays when new SIVs or SIV lineages are described. Using a large set of SIV antigens is important to overcome the impact of the large genetic diversity among primate lentiviruses from different primate species. Overall, the sensitivities of homologous and heterologous antibody detection of the bgp41 ELISA were only slightly lower than for the rpg41 ELISA, 96.0% (86.3–99.5) versus 100 % (95.4–100) but specificities were similar. We compared SIV prevalences in southwest Cameroon (CS5), from two independent surveys over time and each tested by one of both Elisas. We obtained slightly lower global SIV prevalences with the peptide Elisa, but more in detailed analysis, showed that this was only related to different prevalences in mandrills. Since the SIVmnd-2 peptide performed well on the reference panel, it is most likely that these differences are due to the limited number of samples and to possible hunting bias, ex. animals can be derived from the same troop of mandrills, in which prevalences can vary, or from different troops and have an impact on subsequent prevalences.

SIV prevalences in primate bushmeat from different geographic areas

Based on cross-reactivity with HIV antigens, we initially reported that a relative high proportion of primate bushmeat was potentially infected with SIV (Peeters et al., 2002). Subsequently, by using SIV specific Elisa, we showed that SIV prevalences in wild NHP from Cameroon vary between primate species, and that HIV cross-reactivity could either lead to false negative reactions or false positive reactions (Aghokeng et al., 2006). In order to study more in detail SIV prevalences in primate bushmeat, we extended our survey among wild primates and collected additional samples from primate bushmeat at 5 different rural sites throughout southern Cameroon. We also re-analyzed samples from our previous survey according to the geographic origin of samples. We analyzed a total of 2586 samples from 11 different species, 730 as whole blood and 1856 as dried spots onto filter paper to overcome the issues of storage and transportation from remote forest areas to the capital city Yaoundé. The overall SIV prevalence was low, 2.9% (2.3–3.6). Interestingly, differences in overall SIV prevalences were observed among the different collection sites, ranging from 0.0% to up to 5.7%, with a gradient increasing from the southeast to the southwest. We also confirmed significant differences among different species. Compared to other reports, we confirmed high prevalences for *C. tantalus*, *C. torquatus*, *C. neglectus*, *M. sphinx*, and *M. ogouensis* (Bibollet-Ruche et al., 1997; Beer et al., 2001; Souquiere et al., 2001; Takehisa et al., 2001; Aghokeng et al., 2006;). We did not find any evidence of SIV infection in crested monas and in agile and grey-cheeked mangabeys. Given the large number of samples tested for these latter species it is now becoming less likely that these primate species are naturally infected with SIV, at least in the areas we studied. Although it cannot be excluded that these species are naturally infected and that our screening tools are still not broadly sensitive enough to cross-react with SIV-antibodies from these primates. Further investigations are needed to address this issue.

We found a very low SIV infection rate, 1%, for two of the most commonly hunted primate species, *C. nictitans* and *C. cephus* and observed differences according to the geographic origin. For *C. cephus*, prevalences reach up to 7% in Eboumetoum (CS6) but are low or absent in the other sites. For *C. nictitans*, prevalences are highest on market of Yaoundé where the exact origin of samples is not known. Geographic differences among SIV prevalences seem also to exist for *C. guereza* in our study and have previously also been shown clearly for other non-human primate species. Especially, our surveys among more than 800 samples from wild chimpanzee in 19 different sites in southern Cameroon showed that prevalences can vary from up to 30% to absence or low prevalences of SIV infection depending on the geographic region where samples were collected (Van Heuverswyn et al., 2007).

SIV prevalences and risk for cross-species transmission to humans

Interestingly, in addition to the overall low SIV prevalences, absence of SIV infection or low SIV prevalences were observed in the species which are most frequently hunted, i.e. *C. nictitans*, *C. cephus*, and *C. pogonias* in all tested sites. In contrast, the highest prevalences were seen among species which are only present at low proportions as primate bushmeat, i.e. De Brazza monkeys, talapoins, and mandrills. In addition the presence of these species among bushmeat is mainly restricted to the southwestern part of Cameroon, corresponding to their habitats. In the southeast the proportion of agile and grey-cheeked mangabeys represents about 25% of primate bushmeat, but up to now no SIV infection has been documented in them. The differences in prevalences and the low proportions of highly infected species among primate bushmeat could in part explain why no cross-species transmission to humans with these viruses have yet been observed in Cameroon.

However, chimpanzees and gorillas represent also only a relative small proportion of primate bushmeat, but SIVcpz prevalences can reach up to 30% in certain areas and it has to be noted also that in certain areas of central Africa chimpanzees and/or gorillas, are not only hunted for

bushmeat but also for traditional medicine and religion implying direct contact with large volumes of infected blood or large proportions of infected tissues (Meder, 1999). Moreover, the highest prevalences (>30%) of SIVcpz have been observed in chimpanzee populations infected with variants most closely related to HIV-1M and HIV-1N, arguing in favor for the higher likelihood of SIV transmission when prevalences are high (Keele et al., 2006; Van Heuverswyn et al., 2007). Similarly, sooty manbabeyes represent a high proportion of primate bushmeat in West Africa and SIV prevalences are high in this species (>50%), which could explain the high numbers (n=8) of documented cross-species transmissions (Hahn et al., 2000). However, high SIV prevalences are not the only explanation for possible cross-species transmissions or not, because in the same region where SIVsmm was transmitted several times to humans, western red colobus represent together with sooty mangabeys a high proportion of primate bushmeat. The SIV prevalence in this species is also high, 25–50%, but no human infections have yet been detected (Locatelli et al., 2008b).

Molecular evidence of cross-species transmission of other retroviruses from primates to humans has been reported for STLV and SFV but prevalences of these viruses are also high among wild primate populations (Mahieux et al., 1998; Wolfe et al., 2004). Our previous studies in primate bushmeat showed an overall higher STLV prevalence (11%) and high prevalences among some commonly hunted species, e.g. *C. agilis* in south and southeast Cameroon are infected at rates of up to 65% (Cournaud et al., 2004; Liegeois et al., 2008). These differences in prevalences could explain why new HTLV variants and SFV infections were detected among Cameroonian hunters (Wolfe et al., 2004; Mahieux and Gessain, 2005).

Genetic diversity among SIVs

We also showed a high genetic diversity among SIVs and identified new SIVdeb sequences from De Brazza monkeys, which could represent geographic clusters. Previous studies have shown that SIVdeb infection is frequent among wild De Brazza's monkeys and widespread throughout the species habitat (Bibollet-Ruche et al., 2004) from south Cameroon through the Congo basin to eastern Uganda (Kingdon, 1997). Our results here confirm and reinforce the previous observations on high genetic diversity and geographic clustering among SIVdeb from Cameroon and Uganda. However this observation on geographic clustering in Cameroon has to be confirmed on full-length genome characterization of the new SIVdeb strains. Phylogeographic clustering was also observed among SIVcpz in wild chimpanzee populations, with identification of SIVcpz variants most closely related to HIV-1 M in the southeast and those related to HIV-1 N in south central Cameroon (Keele et al., 2006; Van Heuverswyn et al., 2007). Previous studies on SIV genetic diversity and evolution among other non-human primates from sub-Saharan Africa have also reported divergent SIVs from the same primate species. The first report was from mandrill from both sides of the Ogooué river in Gabon that are infected with SIVmnd1 south of the river and with SIVmnd2 north of the river corresponding to north Gabon and southern Cameroon (Souquiere et al., 2001; Takehisa et al., 2001). Recently, we showed that mustached monkeys found in Cameroon are infected with two distinct SIVmus variants, that we designated SIVmus-1 and SIVmus-2, which differ in *gag* and *pol* (but not *env*) to a similar extent as SIVs from different host species. The key difference between SIVmnd and SIVmus epidemiology was that contrary to mandrills that are geographically separated by the Ogooué river, mustached monkeys infected with SIVmus-1 and SIVmus-2 shared approximately the same habitat, with no evidence of geographic separation (Aghokeng et al., 2007). But in both cases, cross-species transmission followed by recombination were identified as playing important role in the emergence of one of the two virus infecting the primate species. In the present study, although the sample collection sites in which we reported the two SIVdeb sub-clades respectively in the southern and the northern parts of southeastern Cameroon (Figure 1) are distant enough, about 300 km apart, to believe

that the habitat of these two De Brazza's monkey populations do not overlap, there is also no evidence that these two populations are geographically isolated.

In summary, prevalences most likely play a role in the transmission of certain SIVs and other simian retroviruses to humans, but viral and host factors are also important to establish an efficient infection and disease. The data from our study on SIV prevalences can thus explain in part the situation in Cameroon. However, additional surveys on SIV sero-prevalences among NHP living in sub-Saharan Africa using large sample sizes are needed to evaluate the potential risks for other zoonotic transmissions because primate species and SIV prevalences can differ. Given the ongoing contacts between infected NHP and African populations through hunting and butchering, together with the increase of the bushmeat trade related to increasing presence of logging concessions it is likely that cross-transmissions are still occurring. Moreover, the socioeconomic changes, which go together with the presence of logging concessions or other industries in remote forest areas (Auzel and Hardin, 2000), suggest that the magnitude of human exposure to SIV has increased, as have the social and environmental conditions that support the emergence and spread of new zoonotic infections. In addition, high HIV prevalences in these remote areas (Laurent et al., 2004) could lead to recombinants between HIVs and SIVs and allow more efficient adaptation and replication in the new host. One major public health implication is that, these SIV strains are not always recognized by commercial HIV-1/HIV-2 screening assays and as a consequence, human infection with such variants can go unrecognized for several years and lead to another epidemic.

Acknowledgments

This work was supported in part by grants from the National Institute of Health (RO1 AI 50529) and the Agence Nationale de Recherches sur le SIDA (ANRS, ANRS 12125). Avelin Aghokeng received a postdoctoral fellowship from the Agence Nationale de Recherches sur le SIDA (ANRS) to conduct this work. We thank the Cameroonian Ministries of Health, Research, and Environment and Forestry and Wildlife, for permission to perform this study, and the staff from project PRESICA for logistical support and assistance in the field. The author is also grateful for the training received at the 14th International Bioinformatics Workshop on Virus Evolution and Molecular Epidemiology, September 2008, Cape Town (<http://www.rega.kuleuven.be/cev/workshop/>).

References

- Abascal F, Zardoya R, Posada D. ProtTest: selection of best-fit models of protein evolution. *Bioinformatics* 2005;21:2104–5. [PubMed: 15647292]
- Aghokeng AF, Bailes E, Loul S, Courgnaud V, Mpoudi-Ngolle E, Sharp PM, Delaporte E, Peeters M. Full-length sequence analysis of SIVmus in wild populations of mustached monkeys (*Cercopithecus cephus*) from Cameroon provides evidence for two co-circulating SIVmus lineages. *Virology* 2007;360:407–18. [PubMed: 17156809]
- Aghokeng AF, Liu W, Bibollet-Ruche F, Loul S, Mpoudi-Ngole E, Laurent C, Mwenda JM, Langat DK, Chege GK, McClure HM, Delaporte E, Shaw GM, Hahn BH, Peeters M. Widely varying SIV prevalence rates in naturally infected primate species from Cameroon. *Virology* 2006;345:174–89. [PubMed: 16257029]
- Auzel, P.; Hardin, R. Colonial history, concessionary politics, and collaborative management of Equatorial African rain forests. In: Bakarr, M.; Da Fonseca, G.; Konstant, W.; Mittermeier, R.; Painemilla, K., editors. *Hunting and bushmeat utilization in the African rain forest*. 2000. p. 21–38.
- Beer BE, Foley BT, Kuiken CL, Tooze Z, Goeken RM, Brown CR, Hu J, St Claire M, Korber BT, Hirsch VM. Characterization of novel simian immunodeficiency viruses from red-capped mangabey from Nigeria (SIVrcmNG409 and -NG411). *J Virol* 2001;75:12014–27. [PubMed: 11711592]
- Bibollet-Ruche F, Bailes E, Gao F, Pourrut X, Barlow KL, Clewley JP, Mwenda JM, Langat DK, Chege GK, McClure HM, Mpoudi-Ngole E, Delaporte E, Peeters M, Shaw GM, Sharp PM, Hahn BH. New simian immunodeficiency virus infecting De Brazza's monkeys (*Cercopithecus neglectus*): evidence for a *cercopithecus* monkey virus clade. *J Virol* 2004;78:7748–62. [PubMed: 15220449]

- Bibollet-Ruche F, Brengues C, Galat-Luong A, Galat G, Pourrut X, Vidal N, Veas F, Durand JP, Cuny G. Genetic diversity of simian immunodeficiency viruses from West African green monkeys: evidence of multiple genotypes within populations from the same geographical locale. *J Virol* 1997;71:307–13. [PubMed: 8985351]
- Clewley JP, Lewis JC, Brown DW, Gadsby EL. A novel simian immunodeficiency virus (SIV_{rl}) pol sequence from the drill monkey, *Mandrillus leucophaeus*. *J Virol* 1998;72:10305–9. [PubMed: 9811781]
- Courgnaud V, Abela B, Pourrut X, Mpoudi-Ngole E, Loul S, Delaporte E, Peeters M. Identification of a new simian immunodeficiency virus lineage with a vpu gene present among different cercopithecus monkeys (*C. mona*, *C. cephus*, and *C. nictitans*) from Cameroon. *J Virol* 2003;77:12523–34. [PubMed: 14610175]
- Courgnaud V, Pourrut X, Bibollet-Ruche F, Mpoudi-Ngole E, Bourgeois A, Delaporte E, Peeters M. Characterization of a novel simian immunodeficiency virus from guereza colobus monkeys (*Colobus guereza*) in Cameroon: a new lineage in the nonhuman primate lentivirus family. *J Virol* 2001;75:857–66. [PubMed: 11134299]
- Courgnaud V, Van Dooren S, Liegeois F, Pourrut X, Abela B, Loul S, Mpoudi-Ngole E, Vandamme A, Delaporte E, Peeters M. Simian T-cell leukemia virus (STLV) infection in wild primate populations in Cameroon: evidence for dual STLV type 1 and type 3 infection in agile mangabeys (*Cercocebus agilis*). *J Virol* 2004;78:4700–9. [PubMed: 15078952]
- Crowther, JR. The ELISA Guidebook. In: Walker, JM., editor. *Methods in Molecular Biology*. Vol. 149. Humana Press; Totowa, NJ: 2001.
- Gao F, Bailes E, Robertson DL, Chen Y, Rodenburg CM, Michael SF, Cummins LB, Arthur LO, Peeters M, Shaw GM, Sharp PM, Hahn BH. Origin of HIV-1 in the chimpanzee *Pan troglodytes troglodytes*. *Nature* 1999;397:436–41. [PubMed: 9989410]
- Gao F, Yue L, White AT, Pappas PG, Barchue J, Hanson AP, Greene BM, Sharp PM, Shaw GM, Hahn BH. Human infection by genetically diverse SIVSM-related HIV-2 in west Africa. *Nature* 1992;358:495–9. [PubMed: 1641038]
- Groves, C. *Smithsonian Series in Comparative Evolutionary Biology*. Smithsonian Institution Press; 2001. *Primate Taxonomy*.
- Hahn BH, Shaw GM, De Cock KM, Sharp PM. AIDS as a zoonosis: scientific and public health implications. *Science* 2000;287:607–14. [PubMed: 10649986]
- Hirsch VM, Olmsted RA, Murphey-Corb M, Purcell RH, Johnson PR. An African primate lentivirus (SIV_{sm}) closely related to HIV-2. *Nature* 1989;339:389–92. [PubMed: 2786147]
- Huelsenbeck JP, Ronquist F. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 2001;17:754–5. [PubMed: 11524383]
- Keele BF, Van Heuverswyn F, Li Y, Bailes E, Takehisa J, Santiago ML, Bibollet-Ruche F, Chen Y, Wain LV, Liegeois F, Loul S, Ngole EM, Bienvenue Y, Delaporte E, Brookfield JF, Sharp PM, Shaw GM, Peeters M, Hahn BH. Chimpanzee reservoirs of pandemic and nonpandemic HIV-1. *Science* 2006;313:523–6. [PubMed: 16728595]
- Kingdon, J. *The kingdon field guide to African mammals*. Press, A., editor. 1997.
- Laurent C, Bourgeois A, Mpoudi M, Butel C, Peeters M, Mpoudi-Ngole E, Delaporte E. Commercial logging and HIV epidemic, rural Equatorial Africa. *Emerg Infect Dis* 2004;10:1953–6. [PubMed: 15550206]
- Liegeois F, Courgnaud V, Switzer WM, Murphy HW, Loul S, Aghokeng A, Pourrut X, Mpoudi-Ngole E, Delaporte E, Peeters M. Molecular characterization of a novel simian immunodeficiency virus lineage (SIV_{tal}) from northern talapoin (*Miopithecus ogouensis*). *Virology* 2006;349:55–65. [PubMed: 16469345]
- Liégeois F, Lafay B, Switzer WM, Locatelli S, Mpoudi-Ngolé E, Loul S, Heneine W, Delaporte E, Peeters M. Identification and molecular characterization of new STLV-1 and STLV-3 strains in wild-caught nonhuman primates in Cameroon. *Virology* 2008;371:405–17. [PubMed: 17976676]
- Liégeois F, Lafay B, Formenty P, Locatelli S, Courgnaud V, Delaporte E, Peeters M. Full-length genome characterization of a novel simian immunodeficiency virus lineage (SIV_{Volc}) from olive Colobus (*Procolobus verus*) and new SIV_{wrcPbb} strains from Western Red Colobus (*Piliocolobus badius badius*) from the Tai Forest in Ivory Coast. *J Virol* 2009;83:428–39. [PubMed: 18922864]

- Locatelli S, Lafay B, Liegeois F, Ting N, Delaporte E, Peeters M. Full molecular characterization of a simian immunodeficiency virus, SIVwrcpbt from Temminck's red colobus (*Piliocolobus badius temminckii*) from Abuko Nature Reserve, The Gambia. *Virology* 2008a;376:90–100. [PubMed: 18442839]
- Locatelli S, Liegeois F, Lafay B, Roeder AD, Bruford MW, Formenty P, Noe R, Delaporte E, Peeters M. Prevalence and genetic diversity of simian immunodeficiency virus infection in wild-living red colobus monkeys (*Piliocolobus badius badius*) from the Tai forest, Cote d'Ivoire SIVwrc in wild-living western red colobus monkeys. *Infect Genet Evol* 2008b;8:1–14. [PubMed: 17916449]
- Mahieux R, Chappey C, Georges-Courbot MC, Dubreuil G, Maucelere P, Georges A, Gessain A. Simian T-cell lymphotropic virus type 1 from *Mandrillus sphinx* as a simian counterpart of human T-cell lymphotropic virus type 1 subtype D. *J Virol* 1998;72:10316–22. [PubMed: 9811783]
- Mahieux R, Gessain A. [New human retroviruses: HTLV-3 and HTLV-4]. *Med Trop (Mars)* 2005;65:525–8. [PubMed: 16555510]
- Ndongmo CB, Switzer WM, Pau CP, Zeh C, Schaefer A, Pieniazek D, Folks TM, Kalish ML. New multiple antigenic peptide-based enzyme immunoassay for detection of simian immunodeficiency virus infection in nonhuman primates and humans. *J Clin Microbiol* 2004;42:5161–9. [PubMed: 15528710]
- Meder A. Gorillas in African culture and medicine. *Gorilla J* 1999;18:11–15.
- Peeters M, Courgnaud V, Abela B, Auzel P, Pourrut X, Bibollet-Ruche F, Loul S, Liegeois F, Butel C, Koulagna D, Mpoudi-Ngole E, Shaw GM, Hahn BH, Delaporte E. Risk to human health from a plethora of simian immunodeficiency viruses in primate bushmeat. *Emerg Infect Dis* 2002;8:451–7. [PubMed: 11996677]
- Posada D, Crandall KA. MODELTEST: testing the model of DNA substitution. *Bioinformatics* 1998;14:817–8. [PubMed: 9918953]
- Sharp PM, Shaw GM, Hahn BH. Simian immunodeficiency virus infection of chimpanzees. *J Virol* 2005;79:3891–902. [PubMed: 15767392]
- Simon F, Souquiere S, Damond F, Kfutwah A, Makuwa M, Leroy E, Rouquet P, Berthier JL, Rigoulet J, Lecu A, Telfer PT, Pandrea I, Plantier JC, Barre-Sinoussi F, Roques P, Muller-Trutwin MC, Apetrei C. Synthetic peptide strategy for the detection of and discrimination among highly divergent primate lentiviruses. *AIDS Res Hum Retroviruses* 2001;17:937–52. [PubMed: 11461679]
- Souquiere S, Bibollet-Ruche F, Robertson DL, Makuwa M, Apetrei C, Onanga R, Kornfeld C, Plantier JC, Gao F, Abernethy K, White LJ, Karesh W, Telfer P, Wickings EJ, Maucelere P, Marx PA, Barre-Sinoussi F, Hahn BH, Muller-Trutwin MC, Simon F. Wild *Mandrillus sphinx* are carriers of two types of lentivirus. *J Virol* 2001;75:7086–96. [PubMed: 11435589]
- Takehisa J, Harada Y, Ndambi N, Mboudjeka I, Taniguchi Y, Ngansop C, Kuete S, Zekeng L, Ibuki K, Shimada T, Bikandou B, Yamaguchi-Kabata Y, Miura T, Ikeda M, Ichimura H, Kaptue L, Hayami M. Natural infection of wild-born mandrills (*Mandrillus sphinx*) with two different types of simian immunodeficiency virus. *AIDS Res Hum Retroviruses* 2001;17:1143–54. [PubMed: 11522184]
- Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* 1997;25:4876–82. [PubMed: 9396791]
- Van Der Kuyl AC, Van Gennep DR, Dekker JT, Goudsmit J. Routine DNA analysis based on 12S rRNA gene sequencing as a tool in the management of captive primates. *J Med Primatol* 2000;29:307–315. [PubMed: 11168820]
- Van Heuverswyn F, Li Y, Bailes E, Neel C, Lafay B, Keele BF, Shaw KS, Takehisa J, Kraus MH, Loul S, Butel C, Liegeois F, Yangda B, Sharp PM, Mpoudi-Ngole E, Delaporte E, Hahn BH, Peeters M. Genetic diversity and phylogeographic clustering of SIVcpzPtt in wild chimpanzees in Cameroon. *Virology* 2007;368:155–71. [PubMed: 17651775]
- Van Heuverswyn F, Li Y, Neel C, Bailes E, Keele BF, Liu W, Loul S, Butel C, Liegeois F, Bienvenue Y, Ngolle EM, Sharp PM, Shaw GM, Delaporte E, Hahn BH, Peeters M. Human immunodeficiency viruses: SIV infection in wild gorillas. *Nature* 2006;444:164. [PubMed: 17093443]
- Van Heuverswyn F, Peeters M. The Origins of HIV and Implications for the Global Epidemic. *Curr Infect Dis Rep* 2007;9:338–346. [PubMed: 17618555]

- VandeWoude S, Apetrei C. Going wild: lessons from naturally occurring T-lymphotropic lentiviruses. *Clin Microbiol Rev* 2006;19:728–62. [PubMed: 17041142]
- Wolfe ND, Prosser TA, Carr JK, Tamoufe U, Mpoudi-Ngole E, Torimiro JN, LeBreton M, McCutchan FE, Birx DL, Burke DS. Exposure to nonhuman primates in rural Cameroon. *Emerg Infect Dis* 2004a; 10:2094–9. [PubMed: 15663844]
- Wolfe ND, Switzer WM, Carr JK, Bhullar VB, Shanmugam V, Tamoufe U, Prosser AT, Torimiro JN, Wright A, Mpoudi-Ngole E, McCutchan FE, Birx DL, Folks TM, Burke DS, Heneine W. Naturally acquired simian retrovirus infections in central African hunters. *Lancet* 2004b;363:932–7. [PubMed: 15043960]
- Yang Z, Rannala B. Bayesian phylogenetic inference using DNA sequences: a Markov Chain Monte Carlo Method. *Mol Biol Evol* 1997;14:717–24. [PubMed: 9214744]

Figure 1.

Collection sites (CS) of NHP DBS throughout southeastern Cameroon. A total of 2586 samples were collected from 11 different primate species. In sites highlighted in blue (CS 1, CS 2, CS 3, CS 4 and CS 5a), samples were collected as dried blood spots (DBS) and stored at ambient temperature. In sites highlighted in yellow, samples were collected as whole blood in a previous survey (Aghokeng et al., 2006) and stored at -20°C .

Figure 2.

Phylogenetic relationships of the newly derived SIV sequences in *pol* to representatives of the other SIV lineages. The newly identified strains in this study are highlighted in blue for the SIV sequences derived from DBS samples and in red for those derived from whole blood samples. Previously published SIV sequences derived from primate bushmeat in Cameroon are indicated in orange and reference strains from the GenBank and other HIV/SIV databases are in black. The unrooted trees were inferred from 305 bp nucleotides. The numbers at nodes are posterior probabilities estimated by the Bayesian method. Only those 90% and above are shown. The scale bar represents 0.1 replacements per site.

Figure 3.

Phylogenetic relationships of SIVdeb from East and South Cameroon and from Uganda. Maximum likelihood trees were inferred from a partial (164 amino acids) Pol protein sequence alignment. The numbers at nodes indicate estimated posterior probabilities (only values of 90% or greater are shown). The scale bar indicates 0.05 substitutions per site.

Table 1

Amino acid sequences of the peptides used to develop the biotinylated SIV-specific ELISAs.

SIV	SIV lineage	gp41 peptide sequences	V3 peptide sequences
SIVcpzAnt	HIV-1/SIVcpz	LAVEKYLRDQQLSLWGCADKVTCT	
SIVsmm	HIV-2/SIVsmm	TAIEKYLDQQAQLNSWGCAFRQVC	
SIVrcm	SIVrcm	TAIEKYLDQSLNFTFGCAWRQVC	
SIVagm-consensus	SIVagm	TALEKYLEDQARLNAWGCAWKQVC	
SIVgsn		SSLEKYLRDQTILQAWGCANRPIC	
SIVmus	SIVgsn/mus/mon	TALEKFVKDQAILNLWGCAWRQIC	
SIVmon		TAVEKFIKDTLLNAWGCAWKAVC	
SIVdeb	SIVdeb	TAIEKYLDQAKLNEWGCAFKQIC	
SIVtal	SIVtal	TALEKYLEDQAKLNSWGCAWKQIC	
SIVsyk	SIVsyk	TALETYLRDQAISNWSGCAFKQIC	
SIVlho-consensus	SIVlho/sun	TAIEEYLDQALLASWGCAWKQVC	
SIVmnd-2	SIVmnd	TALEDYVADQSRLAVWGCSFSQVC	
SIVwrc	SIVwrc	SAIEGFLEDQLKLQWGCELTQVC	
SIVcol	SIVcol ^a	ATIEGYLEEQAKLASIGCANMQIC	GNSSHRNLNTANGAKFYELIPYSGIYGRCT

^aDue to unsuccessful synthesis of SIVcol gp41 peptide, we included corresponding V3 peptide to obtain a more comprehensive ELISA strategy.

Table 2

Panel of reference sera from SIV infected and non-infected non human primate species.

species	common name	SIV (n)	SIV pos (n)	SIV neg
<i>Pan troglodytes</i>	Chimpanzee	SIVcpz	3	1
<i>Cercopithecus nictitans</i>	Greater spot nosed monkey	SIVgsn	6	45
<i>Cercopithecus cephus</i>	Mustached monkey	SIVmus	7	31
<i>Cercopithecus mona</i>	Mona monkey	SIVmon	-	1
<i>Miopithecus ogouensis</i>	Northern talapoin	SIVtal	2	6
<i>Cercopithecus neglectus</i>	De Brazza monkey	SIVdeb	6	2
<i>Cercocebus torquatus</i>	Red capped mangabey	SIVrcm	4	-
<i>Chlorocebus tantalus</i>	African green monkey	SIVagm	2	-
<i>Mandrillus sphinx</i>	Mandrill	SIVmnd-2	10	2
<i>Colobus guereza</i>	Mantled guereza	SIVcol	10	7
Total			50	95

Table 3

Sensitivity and specificity of the bgp41 SIV ELISAs for homologous and heterologous antibody detection.

Species	SIV	Homologous antibody detection				Homologous and heterologous antibody detection			
		SIV positive samples		SIV negative samples		SIV positive samples		SIV negative samples	
	Antigen	N_{pos}/N_{tested}	Sensitivity	N_{pos}/N_{tested}	Specificity	N_{pos}/N_{tested}	Sensitivity	N_{pos}/N_{tested}	Specificity
<i>P. t. troglodytes</i>	SIVcpz	3/3	100.0%	0/1	100.0%	12/50	24.0%	1/95	98.9%
<i>C. nictitans</i>	SIVgsn	6/6	100.0%	0/45	100.0%	22/50	44.0%	0/95	100.0%
<i>C. cephus</i>	SIVmus	7/7	100.0%	0/31	100.0%	37/50	74.0%	0/95	100.0%
<i>C. mona</i>	SIVmon ^a	—/—	—/—	0/1	100.0%	16/50	32.0%	1/95	98.9%
<i>M. ogouensis</i>	SIVtal	1/2	50.0%	0/6	100.0%	25/50	50.0%	0/95	100.0%
<i>C. neglectus</i>	SIVdeb	6/6	100.0%	0/2	100.0%	29/50	58.0%	0/95	100.0%
<i>C. albogularis</i>	SIVsyk ^a	—/—	—/—	—/—	—/—	27/50	54.0%	0/95	100.0%
<i>C. arys</i>	SIVsmm ^a	—/—	—/—	—/—	—/—	32/50	64.0%	0/95	100.0%
<i>C. torquatus</i>	SIVrem	4/4	100.0%	—/— ^a	—/—	9/50	18.0%	0/95	100.0%
<i>C. tantalus</i>	SIVagm	2/2	100.0%	—/— ^a	—/—	26/50	52.0%	0/95	100.0%
<i>M. sphinx</i>	SIVmnd-2	10/10	100.0%	0/2	100.0%	20/50	40.0%	0/95	100.0%
<i>C. lhoesti</i>	SIVlho ^a	—/—	—/—	—/—	—/—	17/50	34.0%	0/95	100.0%
<i>P. badius</i>	SIVwrc ^a	—/—	—/—	—/—	—/—	9/50	18.0%	0/95	100.0%
Total		39/40	97.5% (87.1 – 99.6) ^b	0/88	100.0% (95.8 – 100.0) ^b	48/50	96.0% (86.5 – 98.9) ^b	2/95	97.9% (92.6 – 99.4) ^b

^a No reference sample available

^b 95% confidence intervals

Table 4

The number and percentages of SIV positive samples per species and per site. Samples collected at CS1 to CS5a were collected as dried blood spots, and the remaining (CS5b to CS7) were whole blood samples as previously reported. At CS5, samples have been collected as DBS in 2004 (CS5a), and as whole blood in 2001(CS5b)

Species	Common name	CS1	CS2	CS3	CS4	CS5a	CS5b	CS6	CS7	Total <i>Npos/Ntested</i>
		Yokadouma <i>Npos/Ntested</i>	Bertoua <i>Npos/Ntested</i>	Nanga-Eboko <i>Npos/Ntested</i>	Nord Sanaga <i>Npos/Ntested</i>	Ebolowa <i>Npos/Ntested</i>	Ebolowa-old <i>Npos/Ntested</i>	Eboumetoum <i>Npos/Ntested</i>	Yaounde <i>Npos/Ntested</i>	
<i>Cercopithecus cephus</i>	Mustached monkey	0/133 (0.0%)	1/105 (1.0%)	2/217 (0.9%)	0/17 (0.0%)	0/175 (0.0%)	0/81 (0.0%)	6/82 (7.3%)	0/54 (0.0%)	9/864 [1.0% (0.5–1.9) ^a]
<i>Cercopithecus mitatus</i>	Greater spot nosed monkey	0/296 (0.0%)	0/73 (0.0%)	1/187 (0.5%)	0/18 (0.0%)	0/99 (0.0%)	0/44 (0.0%)	0/30 (0.0%)	8/112 (7.1%)	9/859 [1.0% (0.5–1.9)]
<i>Cercopithecus pogonias</i>	Crested mona	0/87 (0.0%)	0/23 (0.0%)	0/72 (0.0%)	0/12 (0.0%)	0/32 (0.0%)	0/16 (0.0%)	0/21 (0.0%)	0/30 (0.0%)	0/293 [0.0% (0.0–0.1)]
<i>Cercocercops agilis</i>	Agile mangabey	0/81 (0.0%)	0/2 (0.0%)	–/–	–/–	–/–	–/–	0/93 (0.0%)	0/6 (0.0%)	0/182 [0.0% (0.0–0.2)]
<i>Lophoceros albigena</i>	Grey-cheeked mangabey	0/122 (0.0%)	0/2 (0.0%)	0/2 (0.0%)	0/3 (0.0%)	–/–	–/–	0/27 (0.0%)	0/12 (0.0%)	0/168 [0.0% (0.0–0.2)]
<i>Colobus guereza</i>	Mantled guereza	0/16 (0.0%)	–/–	0/1 (0.0%)	–/–	0/8 (0.0%)	–/–	4/26 (15.4%)	10/27 (37.0%)	14/78 [17.9% (11.0–27.9)]
<i>Miopithecus ogouensis</i>	Northern talapoin	–/–	–/–	–/–	–/–	7/37 (18.9%)	1/7 (14.3%)	0/6 (0.0%)	2/10 (20.0%)	10/60 [16.7% (9.3–28.0)]
<i>Cercopithecus neglectus</i>	De Brazza monkey	0/3 (0.0%)	1/1 (100.0%)	1/6 (16.7%)	–/–	2/5 (40.0%)	–/–	7/15 (46.7%)	4/7 (57.1%)	15/37 [40.5% (26.3–56.5)]
<i>Mandrillus sphinx</i>	Mandrill	–/–	–/–	–/–	–/–	2/11 (18.2%)	5/7 (71.4%)	–/–	3/9 (33.3%)	10/27 [33.3% (21.5–55.7)]
<i>Cercocercops torquatus</i>	Red capped mangabey	–/–	–/–	–/–	–/–	3/7 (42.9%)	3/3 (100.0%)	–/–	0/2 (0.0%)	6/12 [50.0% (25.3–74.6)]
<i>Chlorocebus tantalus</i>	African green monkey	–/–	–/–	1/2 (50.0%)	1/1 (100.0%)	–/–	–/–	–/–	1/3 (33.3%)	3/6 [50.0% (18.7–81.2)]
Total		0/738 [0.0% (0.0–0.5) ^a]	2/206 [1.0% (0.2–3.4)]	5/487 [1.0% (0.4–2.3)]	1/51 [2.0% (0.3–10.3)]	14/374 [3.7% (2.2–6.1)]	9/158 [5.7% (3.0–10.4)]	17/300 [5.7% (3.5–8.8)]	28/272 [10.3% (7.2–14.4)]	76/2586 [2.93% (2.3–3.6)]

^a95% confidence intervals